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FGFR2 Mutation in Clinically Nonclassifiable Autosomal Dominant Craniosynostosis With Pronounced Phenotypic Variation

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We describe a mutation in the FGFR2 gene in affected members of a large family with inherited autosomal dominant craniosynostosis. The mutation is a G1044A transition at codon 344 of exon B of the gene and results in abnormal splicing of the FGFR2 transcript. The phenotypic effect of the mutation varies greatly. It ranges from minor anomalies such as slight hypertelorism and maxillary hypoplasia to severe manifestations such as brachycephaly and dolichocephaly. The severe cases required surgery because of increased intracranial pressure. The patients cannot be assigned clinically to one of the known craniosynostotic syndromes with mutations in FGFR2, e.g., Crouzon, Pfeiffer, or Jackson-Weiss. This study demonstrates that FGFR2 mutations can result in a spectrum of craniofacial abnormalities even within one family. The known eponymic syndromes of Crouzon, Pfeiffer, or Jackson-Weiss only describe phenotypic extremes of this spectrum. Therefore, the clinical classification should be abandoned and replaced by a molecular one such as "FGFR-associated craniosynostosis syndromes." © 1996 Wiley-Liss, Inc.

KEY WORDS: **craniosynostosis, maxillary hypoplasia, brachycephaly, dolichocephaly, FGFR2 mutation**

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INTRODUCTION

The term craniosynostosis refers to a range of skull deformities that results from the premature fusion and abnormal development of the calvarial sutures. Craniosynostosis occurs frequently in combination with other anomalies of bone differentiation, notably of the hands and feet. At least 100 syndromes have been described with craniosynostosis [Cohen, 1986]. Several autosomal dominant craniosynostosis syndromes were clinically delineated. They are best known by their eponyms and include the syndromes of Apert, Crouzon, Pfeiffer, and Jackson-Weiss [Gorlin et al., 1990]. Apert syndrome is characterized by craniosynostosis and severe syndactyly. The most prominent signs of Crouzon syndrome are orbital proptosis and hypertelorism in addition to craniosynostosis. Hands and feet are usually not affected. The craniosynostotic syndrome of Pfeiffer is clinically diagnosed by the occurrence of broad thumbs and toes. Foot anomalies are typical findings in Jackson-Weiss syndrome.

During the last 2 years, the molecular basis of the autosomal dominant syndromes of Apert, Crouzon, Pfeiffer, and Jackson-Weiss was elucidated [Müller and Graeber, 1996]. Mutations in the fibroblast growth factor receptor 2 (FGFR2) gene on chromosome 10 (10q26) can give rise to all four syndromes [Jabs et al., 1994; Reardon et al., 1994; Lajeunie et al., 1995; Rutland et al., 1995; Wilkie et al., 1995; Steinberger et al., 1995, 1996]. In addition, a phenotypically indistinguishable subgroup of Pfeiffer syndrome can be caused by mutations in FGFR1 on chromosome 8 (8p) [Muenke et al., 1994].

All mutations known to date in the FGFR2 gene have been found in exons 5 and 7 that encode the Ig-like chain III (IIIa and IIIc) of FGFR2. Of those mutations, several are observed in more than one condition. Thus, identical mutations were detected in Crouzon and Pfeiffer [Rutland et al., 1995; Gorry et al., 1995; Lajeunie et al, 1995; Meyers et al., 1996; Oldridge et al., 1995] and in Crouzon and Jackson-Weiss [Gorry et al., 1995; Oldridge et al., 1995; Meyers et al., 1996]

syndromes. In contrast, specific mutations have been described in Apert syndrome [Wilkie et al., 1995]. These mutations occur at the adjacent amino acid positions 252 and 253 of IgIIIa of FGFR2. The findings show that Pfeiffer, Crouzon, and Jackson-Weiss syndromes are not distinguishable at the molecular level and that Apert syndrome is probably caused by allelic mutations.

Here we describe a family with autosomal dominant craniosynostosis that could not be clinically assigned to any of the well-established craniosynostosis syndromes. Analysis of FGFR2 in this family demonstrated a mutation in codon 344 of exon 7 of FGFR2 that was previously observed in Crouzon syndrome [Jabs et al., 1994; Reardon et al., 1994].

CLINICAL REPORTS

The family described is of Turkish origin. The pedigree is shown in Figure 1. All members were examined clinically. Limbs of all affected persons were normal, with the exception of I-1 who had broad great toes. Asterisks in Figure 1 indicate those individuals who participated in molecular genetic testing.

I-1 (Fig. 2a)

A 62-year-old healthy man with mild maxillary hypoplasia as the only craniofacial abnormality. He was the only family member with distinctly broad great toes (Fig. 3).

II-2 (Fig. 2b)

A 32-year-old son of I-1 with pronounced craniofacial findings including brachycephaly, exorbitism, hypertelorism, and maxillary hypoplasia. He has had severe chronically recurring headaches since childhood. At age 7 his right eye became amaurotic due to compression of the optic nerve. He was operated on at ages 16 and 17 for strabismus divergens. At age 21 he had an osteoplastic operation of the right infraorbital region. At this age, increasing intracranial pressure and concomitant deterioration of vision of his left eye necessitated a trepanation for decompression of the optic nerve. At age 31 a ventriculo-peritoneal shunt was placed to alleviate increasing intracranial pressure. X-ray analysis at that time demonstrated generalized impressiones digitatae of the skull and excavation of the sella turcica as the result of chronic intracranial pressure.

II-3 (Fig. 2c)

A healthy 30-year-old son of I-1 with maxillary hypoplasia as the only craniofacial abnormality.

II-6 (Fig. 2d)

A healthy 23-year-old daughter of I-1 with mild hypertelorism and left exorbitism.

III-1 (Fig. 2e)

A 15-year-old son of II-2 with severe craniofacial abnormalities including brachycephaly, hypertelorism, retrusion of the supraorbital ridges, and maxillary hypoplasia. Ophthalmologic examination showed myopia (-1), astigmatism, and mild strabismus divergens. He suffers chronically recurring headaches. Radiography at age 14 demonstrated complete ossification of the cranial sutures. Slight excavation of the sella turcica was interpreted as a result of slightly in-

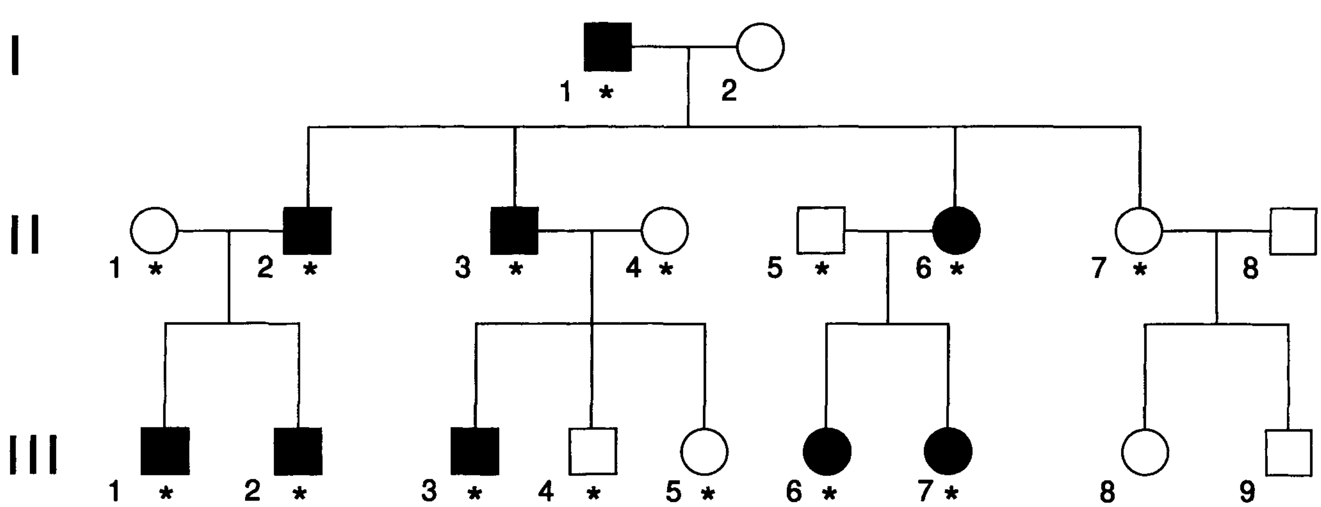

Fig. 1. Pedigree of the family studied. All individuals were examined clinically. Those marked by asterisks were also investigated for mutations in FGFR2.

creased intracranial pressure. Furthermore, lateral divergence of the orbitae was noted.

III-2 (Fig. 2f)

A 10-year-old son of II-2 with severe dolichocephaly, acrocephaly, and pronounced maxillary hypoplasia in the absence of clinical complaints. Radiography documented synostosis of the sagittal and lambdoid sutures but no premature closure of the coronal suture. Parietal impressiones digitatae and slight dilatation of the fourth ventricle were observed.

III-3 (Fig. 2g)

A 4-year-old son of II-3 with brachycephaly and mild hypertelorism. At age 3 radiography showed premature closure of all cranial sutures. Generalized impressiones digitatae were observed as a result of increased intracranial pressure. Craniotomy was performed at age 3 due to bilateral papilloedema.

III-6 (Fig. 2h)

A 4-year-old healthy daughter of II-6 with hypertelorism and no additional craniofacial abnormalities.

III-7 (Fig. 2i)

A 3-year-old healthy daughter of II-6. Mild hypertelorism is the only craniofacial abnormality observed in this patient.

MATERIALS AND METHODS

EDTA blood was obtained from the individuals marked by asterisks in the pedigree of Figure 1, and DNA was extracted according to standard procedures. Primers used for the amplification of exon B (exon 7) of FGFR2 were described previously [Steinberger et al., 1995]. The protocols for amplification of this exon by polymerase chain reaction (PCR) are given in Steinberger et al. [1996]. Single strand conformation polymorphism (SSCP) analysis [Orita et al., 1989] of the amplification products was done as described previously [Kostrzewa et al., 1994]. Amplification products from all patients and their parents were sequenced directly with sequencing grade Taq polymerase (Promega) according to standard procedures [Ausubel et al., 1994]. In addition, amplification products from some patients were cloned into pBluescript for sequencing. Both strands were sequenced in all cases.

RESULTS

Individuals marked by asterisks in the pedigree of Figure 1 were analyzed for mutations in exon B of the FGFR2 gene. Initial screening by SSCP showed band shifts in all affected but not in unaffected members of the family (not shown). Subsequently, exon B was sequenced in these individuals. A G→A transition at position 1044 (codon 344) was observed in affected but not in unaffected persons (not shown). Two persons (III-6 and III-7) with this mutation had mild hypertelorism as the only craniofacial sign. The mutation detected in this family does not result in a change of the amino acid alanine at this position but creates a cryptic splice site (see below).

DISCUSSION

The mutation observed in this family at position 344 of the FGFR2 gene was described previously in Crouzon syndrome [Reardon et al., 1994]. It was shown that the G→A transition at this location results in abnormal splicing of the FGFR2 transcript and a deletion of 17 amino acids in the IgIIIc domain of FGFR2 [Del Gatto and Breathnach, 1995; Li et al., 1995].

The craniosynostosis described here cannot be assigned to any of the known autosomal dominant syndromes by clinical criteria. Unlike in Pfeiffer syndrome, broad thumbs were not observed in any patients. Furthermore, only one patient (I-1) had broad great toes. In contrast to Jackson-Weiss syndrome, no abnormalities of feet were observed in most patients. The only patient with broad great toes did not display medial deviation of this digit, which is a common finding in Jackson-Weiss syndrome [Gorlin, 1990]. All other affected members of this family also did not display abnormal positions of the great toes. Finally, pronounced exorbitism, the hallmark of Crouzon syndrome, was not found in these patients. Mild exorbitism was observed in only two individuals.

Expression of the syndrome varied greatly. Despite the same mutation in FGFR2, several persons were healthy and had only mild facial findings such as slight hypertelorism and maxillary hypoplasia. However, others were severely affected, and their craniosynostoses caused increased intracranial pressure with complications such as severe headaches and compression of the optic nerve. Even the pattern of premature closure of the cranial sutures varied among patients. Different timing and location of abnormal sutural development resulted in phenotypic extremes such as dolichocephaly in one patient (III-2) and brachycephaly in another patient (II-2).

The present study is another example of the variability of the phenotypic effects of identical mutations in FGFR2. For example, identical mutations were described previously in both Crouzon and Jackson-Weiss [Gorry et al., 1995; Park et al., 1995] and Crouzon and Pfeiffer [Rutland et al., 1995] syndromes. These findings indicated that the clinically distinct syndromes are extremes of a spectrum of cranial malformations with the eponymous syndromes at opposite ends of this spectrum. This assumption is now born out by findings within one family of highly variable craniofacial manifestations that cannot be assigned to any known syndrome.

Great phenotypic variability was also found in an autosomal dominant craniosynostosis syndrome that is not caused by a mutation in a FGF receptor gene, i.e., craniosynostosis, type 2 (formerly Boston type)

Fig. 2. (**a–i**): Spectrum of craniofacial manifestations in the patients studied. For further details, see Clinical Reports.

[Müller et al., 1993; Warman et al., 1993]. In one large family, a mutation was found in the homeotic gene *MSX2* [Jabs et al., 1993]. The phenotype of affected members varied greatly and included forehead retrusion, frontal boss, turricephaly, and clover leaf skull. Evidently, several other genetic and nongenetic factors modify the differentiation of the calvarial sutures. Thus, one may speculate that the timing and level of expression of additional growth factors (EGF, PDGF, IGF, TGF-β), hormones (e.g., thyroxin), and their receptors modulate pathways crucial to cranial differentiation.

In conclusion, the present study further emphasizes the need to abandon the classification of some autosomal dominant craniosynostosis syndromes according to eponyms, e.g., Crouzon, Pfeiffer, and Jackson Weiss.

Fig. 2. (*Continued.*)

Terms such as "FGFR-associated craniosynostosis syndromes" are preferable because they also account for clinically "intermediate" forms of some autosomal dominant craniosynostoses.

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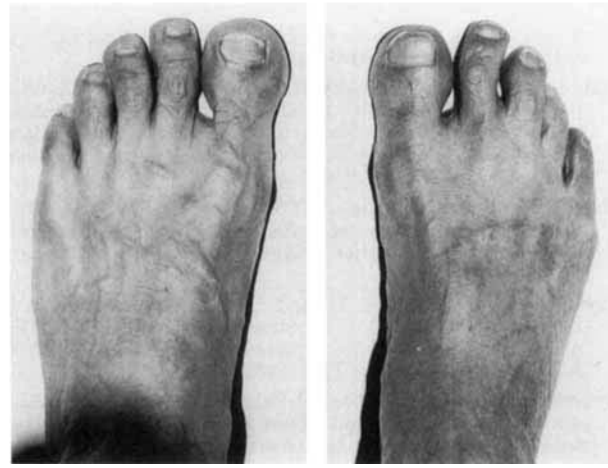

Fig. 3. Feet of patient I-1. Note the broadened great toe.

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